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Immunoglobulin profiles of the chronic antibody response: discussion in relation to brucellosis infections

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Summary

The use of a sensitive and quantitative radioimmunoassay has enabled much finer dissection of the immunoglobulin-antibody profiles for an individual immune response. The kinetics of the response are considered in relation to the switch from IgM to IgG antibody production. In the domestic fowl, the kinetics of this switch varied with different antigens: whereas the response to a thymus-dependent antigen proceeded through a brief 19S response to a declining 7S response, the response to a thymus-independent antigen failed to switch from 19S to 7S for several weeks and consisted of repeated excursions of 19S antibodies. When injected intravenously and simultaneously, *Salmonella adelaide* O (killed) organisms (thymus-independent) and sheep red cells (thymus-dependent) interact so that the response to the latter fails to switch from 19S to 7S and consists of repeated excursions of 19S antibody. The changed character of the sheep red cell response is interpreted as being due to lack of 7S antibody. Passive antibody to either sheep red cells or to *S. adelaide* produced an inhibition of the sheep red rell response so that only one excursion of 19S antibody was observed.

The use of the radio-immunoassay enables an independent measurement of all IgM, IgG and IgA antibody to the surface antigen of *Brucella obortus*. The test, when applied to forty-six sera from individuals with various types of brucellosis, successfully detected antibody in many instances in which conventional serological tests were negative, and such antibody (if IgM) was associated with acute or (if IgG or IgA) with chronic cases of brucellosis. The radioassay test should prove highly valuable effectively to eliminate, in individual patients, the diagnosis of brucellosis based on the inability of conventional tests to detect significant antibody levels.

IN a chronic disease like brucellosis, serology comes to occupy a dominant role in diagnosis since the bacteriologist too often fails to isolate the causal organism from blood, or from marrow obtained by sternal puncture, or from liver obtained at biopsy. Moreover, it has been postulated (Anderson *et al.*, 1964; Reddin *et al.*, 1965; Coghlan and Weir, 1967) that a determination of the immunoglobulin class of *Brucella* antibody present in serum allows determination of the stage of the disease, whether acute, subacute or chronic. Up to the present, the estimation of antibody to *B. abortus* in terms of immunoglobulin class has been deduced for clinical diagnostic purposes from the results of direct and indirect agglutination tests, in presence and absence of 2-mercaptoethanol and by the complement fixation test (Kerr *et al.*, 1966a, b; 1968). In the present approach (Parratt *et al.*, 1977) antibody in each of the three classes IgM, IgG and IgA has been determined directly by the method of radio-immunoassay; but before the results obtained in *Brucella* infections in man are discussed, consideration must be given to the control processes which determine the sequential production of the different immunoglobulin classes of antibody.

The response of man to all antigenic stimuli may involve the subsequent production of antibody in any or all of the immunoglobulin forms IgM, IgG, IgA, IgD and IgE. At present it is not understood why this antibody response needs to be so complex, although it is known that all these molecular forms of antibody have different heavy chains which confer different biological activities. It seems clear that evolutionary progress among the higher vertebrates has coincided with the progressive development of more immunoglobulin classes or subclasses of antibody. The first immunoglobulin class to be made in any immune response is IgM and the biosynthesis of other immunoglobulins is directly dependent on this prior IgM response. The clearest evidence of this is that antibody specific for the μ chain of IgM, injected into neonatal mice (Lawton *et al.*, 1972; Manning and Jutila, 1972a, b; Murgita, Mattioli and Tomasi, 1973), or into chick embryos in the egg (Kincade *et al.*, 1970; Kincade and Cooper, 1971) or used in lymphoid cultures *in vitro* (Pierce, Solliday and Asofsky, 1972) causes suppression of subsequent IgM production and also of other

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immunoglobulins. The injection of antibody against the γ chain of chicken 7S Ig (IgY or IgG) will prevent subsequent production of IgG and IgA but allow production of IgM and the later switch from IgM to IgG occurs within the same plasma cell derivatives (plasmacytes). Although the data, from chickens, of Kincade and Cooper (1971) support the hypothesis that IgA production depends necessarily on a similar later IgG→IgA switch, this was not borne out by the data obtained by Martin and Leslie (1974) in chickens and by the *in vitro* data of Pierce *et al.* (1972). All of these experiments supported the possibility of a direct IgM→IgA switch. The most likely explanation for the effects of anti-μ antiserum on the immune system is a reaction with IgM receptors on the surface of virgin precursor B cells, including those which would eventually give rise to IgA and IgG producers. Consideration of the immunoglobulin composition of sera must take account of immunoglobulin turnover. In man, IgG has the longest half-life (23 days approximately at a normal serum level of IgG) as compared with 6 days for IgA and 5 days for IgM. IgG will, therefore, tend to build up and persist, relative to other immunoglobulins, in the sera from chronic infections.

What controls the switch from one immunoglobulin class to the next? In the adult antibody response it has long been established that specific antibody can interfere with the production of the *same specificity* of antibody (Uhr and Möller, 1968). This demonstration usually involves the injection of an antiserum (principally this is IgG antibody from an hyperimmune serum) before or at the same time as the injection of antigen. This suppresses both IgM and IgG responses, and has been shown to do this in a variety of animal species. More recently, the same suppressive action has been shown for IgA and IgE antibodies (Strannegard and Belin, 1970; Ishizaka and Okudaira, 1972).

The suggestion is that not only are the antibody responses IgM, IgG, IgA and IgE linked in succession but that negative feedback loops exist so that antibody of one class can suppress its own synthesis; the antibody of succeeding classes, expecially IgG, can exert feedback control on antibody of the previous immunoglobulin forms. Clearly there are very many possibilities, but in order to make this presentation tolerable, let us limit consideration to IgM and IgG, with lesser attention to IgA.

Many sources state that both IgM and IgG antibodies have a suppressive action. In the mouse response to sheep erythrocytes (a thymus-dependent antigen at least at low antigen dosage), purified 19S antibody consistently increased the primary response. Antibody of the same specificity, which was 7S, consistently suppressed the response (Henry and Jerne, 1967); and these authors attributed the suppressive effects of 19S, described by others, as due to varying degrees of contamination of the 19S preparations with 7S antibody. Others have found that while a really high dose of 19S antibody is suppressive, lower doses are able to enhance the antibody response (Möller and Wigzell, 1965). The present author's own experiments in the domestic fowl, have consistently confirmed the ability of IgM antibody to produce positive rather than negative feedback. Figure 1 shows the results of increasing doses of IgM antibody given at the same time as the antigenic stimulus: all doses produced an increase in plaque numbers at 6 days of a primary response. It was also found that IgM antibody generally enhanced the response when injected over a range of different times relative to the antigen injection (Fig. 2). Contrariwise, the 7S antibody was always suppressive of the IgM plaque-response, being strongly so when given early in the response.

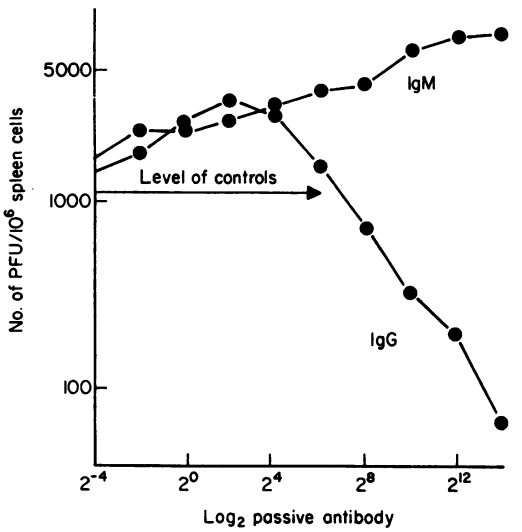

FIG. 1. Effect of increasing amounts of 7S, IgG or 19S, IgM antibody given intravenously to a young fowl on the antibody response at 6 days as judged by the counts of plaque-forming units (PFU) in 10^6 spleen cells. *Note* that all doses of IgM antibody enhanced the response, whereas low doses of IgG antibody enhanced but higher doses produced increasing depression of the response. (Data from experiments with K. H. Nielsen.)

The ability of 7S antibody to produce negative feedback is governed by the avidity of the antibody produced. During the progress of an immunological response there is a progressive rise of antibody avidity, at least if the antigen dose is maintained low. High avidity antibodies are more competent to suppress the immunological response than low avidity antibody (Walker and Siskind, 1968). It is

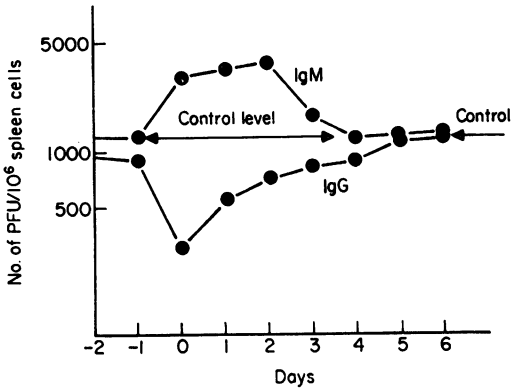

FIG. 2. Effect of a single dose of antibody (7S, IgG or 19S, IgM) given intravenously to a young fowl at various times from 1 day before to 6 days after an intravenous injection of 2 mg human serum albumin (antigen). The ordinate expressed the count of plaque-forming units from 10^6 spleen cells at 6 days after antigen. *Note* that 19S, IgM antibody always enhanced and 7S, IgG antibody always depressed the immune response. (Data from experiments with K. H. Nielsen.)

easy with 7S IgG antibody to suppress the 19S IgM primary response; relatively easy to suppress with hyperimmune serum the 7S IgG primary response; but difficult to suppress the 7S IgG secondary response.

So far, the results quoted relate to thymus-dependent antigens. Thymus-independent antigens are generally regarded as stimulating B cells directly (without aid from macrophages or T-cells) and leading to responses which, at least initially, are exclusively 19S antibody. T-independent immunogens give us the opportunity to study antibody homoeostasis in the absence of 7S antibody. Figure 3 shows the response of individual chickens to an injection of 10^9 heat-killed *Salmonella adelaide*. The antibody which localizes to the surface of these bacilli (mainly anti-O) was estimated by a radioimmunoassay in which IgM or IgG antibody was measured independently of 7S antibody. It can be seen that the resulting IgM response is a cyclical sequence of several peaks of decreasing magnitude. They repeat every 10 days for four cycles before they become dys-synchronous in different animals. During this period of 19S antibody peaks, 7S antibody is undetectable but increases slowly from about 40 days.

The explanation of this antibody profile appears to depend on the following. The antigen generates a 19S antibody response which eventually reaches sufficient concentration to exert negative feedback. However, since there is no shift to 7S antibody and 19S antibody has a very short duration (half-life of 18 hr), suppression is temporary; also, since bacterial lipopolysaccharide is persistent within the tissues, another cycle of 19S antibody is irritated, and so on until 7S antibody makes its appearance in sufficient amounts to secure permanent negative feedback of the response. As would be expected, the cycles of 19S antibody can be brought to a

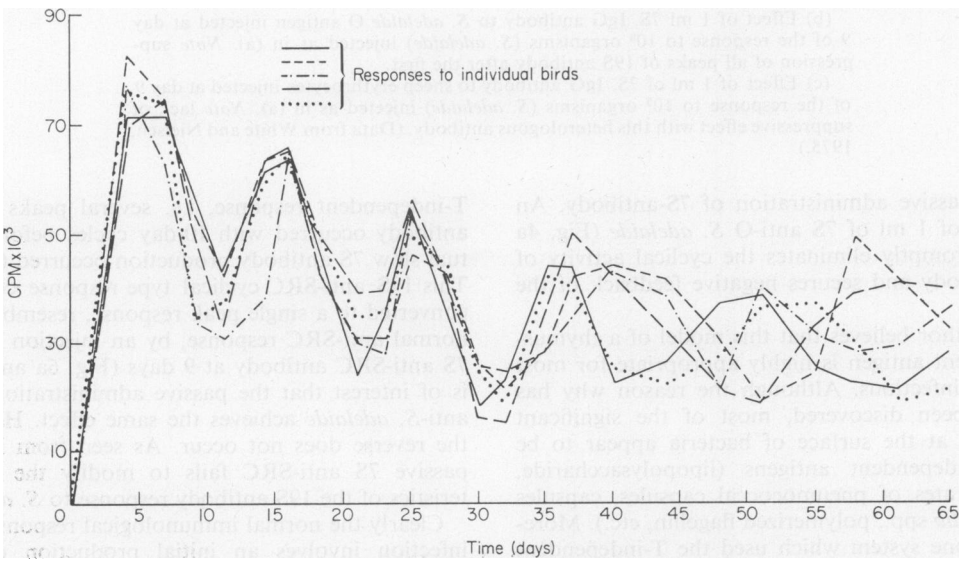

FIG. 3. Antibody responses of individual adult fowl to 10^9 *Salmonella adelaide* O micro-organisms injected i.v. 19S antibody levels were estimated by radio-assay in the five birds at times up to 65 days. *Note* the cyclical response of 19S antibody with peaks at days 4–7, 14–16, 25 and 35–37 days. (Data from White and Nielsen, 1975.)

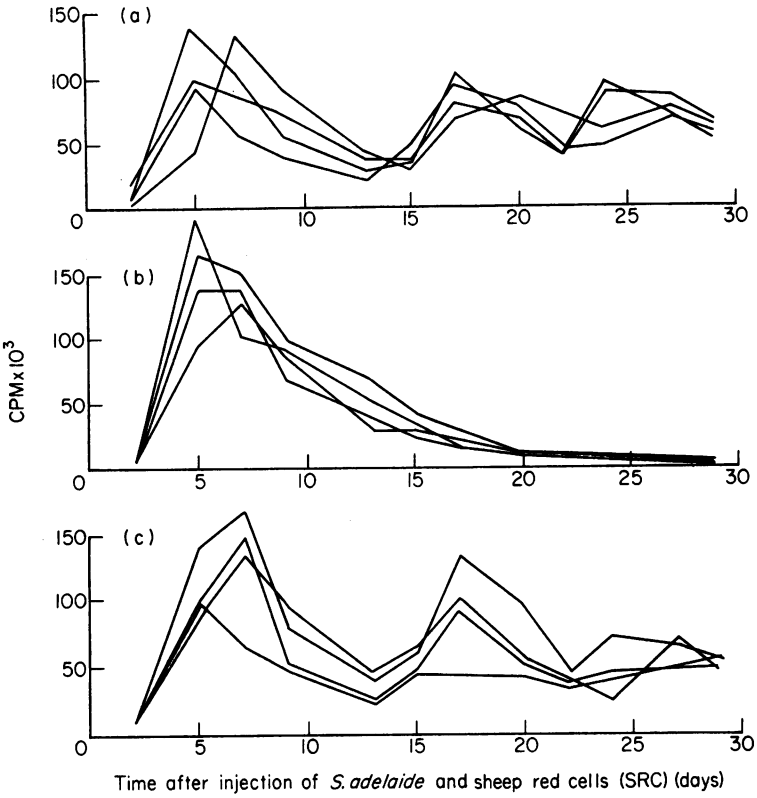

FIG. 4. (a) Antibody responses of individual adult fowl to an injection (day 0) of 10^9 *Salmonella adelaide* O organisms. *Note* cyclical response of 19S antibody, persisting up to 30 days.

(b) Effect of 1 ml 7S, IgG antibody to *S. adelaide* O antigen injected at day 9 of the response to 10^9 organisms (*S. adelaide*) injected as in (a). *Note* suppression of all peaks of 19S antibody after the first.

(c) Effect of 1 ml of 7S, IgG antibody to sheep erythrocytes injected at day 9 of the response to 10^9 organisms (*S. adelaide*) injected as in (a). *Note* lack of suppressive effect with this heterologous antibody. (Data from White and Nielsen, 1975.)

halt by passive administration of 7S-antibody. An injection of 1 ml of 7S anti-O *S. adelaide* (Fig. 4a and b) promptly eliminates the cyclical activity of 19S antibody and secures negative feedback of the response.

The author believes that this model of a thymus-independent antigen is highly appropriate for most bacterial infections. Although the reason why has not yet been discovered, most of the significant molecules at the surface of bacteria appear to be thymus-independent antigens (lipopolysaccharide, carbohydrates of pneumococcal capsules, capsules of *Klebsiella* spp., polymerized flagellin, etc.). Moreover, in one system which used the T-independent *S. adelaide* together with T-dependent sheep erythrocytes (SRC), the agglutinin response to the latter eventually assumed all the characteristics of a T-independent response, i.e. several peaks of 19S antibody occurred with 10-day cycles before eventual slow 7S antibody production occurred (Fig. 5). This 19S anti-SRC cyclical type response could be converted to a single peak response, resembling the normal anti-SRC response, by an injection of 1 ml 7S anti-SRC antibody at 9 days (Fig. 6a and b). It is of interest that the passive administration of 7S anti-*S. adelaide* achieves the same effect. However, the reverse does not occur. As seen from Fig. 4c, passive 7S anti-SRC fails to modify the characteristics of the 19S antibody response to *S. adelaide*.

Clearly the normal immunological response to an infection involves an initial production of IgM antibody followed by IgG antibody, and on the basis of the foregoing arguments the appearance of the latter determines the decline of IgM antibody

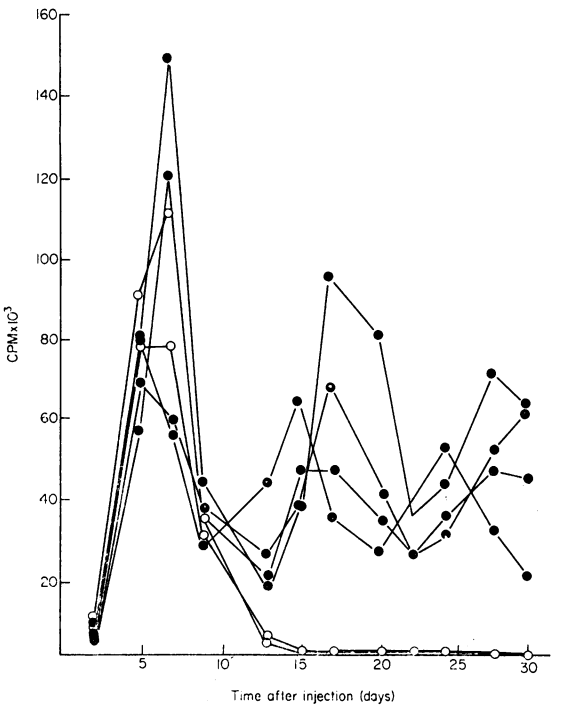

FIG. 5. Effect of an injection of 10^9 *Salmonella adelaide* on the serum antibody response, as detected by radio-assay of 19S antibody, to the simultaneous injection of 10^{10} sheep red cells. Adult fowl were injected at day 0 and serum antibody levels followed for 30 days. *Note* that the simultaneous injection of the bacillary antigen has modified the response to sheep red cells so that it fails to terminate at 15 days and goes through further cycles of 19S antibody production. ○———○ 19S antibody to SRC in chicken injected with SRC. ●———● 19S antibody to SRC in chickens injected with SRC+S. *adelaide*. (Data from White and Nielsen, 1975.)

levels. But this IgM→IgG antibody switch can be delayed, e.g. by T-independent antigens and by an effect of T-independent antigens on the immune mechanism of T-dependent antigens. Decomplementation of the bird by treatment with cobra venom also delays the switch of IgM to IgG for the T-dependent SRBC (Nielsen and White, 1974; White and Nielsen, 1975).

The same type of radio-assay has been applied (Nielsen, Parratt and White, 1973) to the determination of the IgM, IgG and IgA types of antibody in human brucellosis. The assay has the great advantage over other methods of being a primary assay, i.e. it detects any antibody with the ability to combine with the surface antigens of *Brucella* organisms. It thus avoids all the distractions and complications of incomplete or blocking antibody, a notorious component of *Brucella* antisera.

The past decade has witnessed the gradual build-up of an increasingly complicated battery of tests for *Brucella* antibody; direct agglutination, agglutination after addition of anti-human globulin, agglutination after 2-mercaptoethanol, and the complement fixation test. The results of all these have been used to deduce the presence of antibody which is IgM, IgG or IgA. This is only possible in certain instances since IgM, IgA and IgG all give rise to direct agglutination (Wilkinson, 1966), both IgM and IgG can achieve complement fixation, and the relative proportion of individual immunoglobulins which contribute to a titre may be obscure.

The basis of the radio-immunoassay (Nielsen, *et al.*, 1973; Parratt *et al.*, 1977) depends upon the use of a heavy suspension of *Brucella* organisms, which represents an excess of antigen which can absorb all of the specific *Brucella* antibody in 50 μl of serum. After adequate washing, an excess of ^{125}I-labelled sheep anti-human IgG (or IgM or IgA) is added. The amount of uptake of the latter, after washing, measures the antigen-combining power of the serum. Each anti-human Ig must be specific for one of the heavy chains of IgM, IgG or IgA. Also each serum must be tested for rheumatoid factor (RF) since if this is present it will elevate falsely the recorded IgM antibody. In the case of sera with positive RF, these can be absorbed before assay with aggregated human IgG.

In acute cases of brucellosis IgM antibody would be expected in the serum without much IgG or IgA antibody. If IgG has appeared in substantial amounts and produced negative feedback of the immunological response, the IgM antibody would be expected to fall to a low level, and it can be assumed that the chronic stage of a normally evolving immunological response has been reached. In Table 1, cases 1, 2 and 3 fit with the foregoing concept of acute brucellosis. Radioactivity counts above 40×10^3 are regarded as significant for the presence of antibody. Thus, case 2 with 3 weeks pyrexia has high IgM antibody with low IgG antibody. Presumably cases 1 and 3 are approaching the sub-acute phase of disease since they already have substantial levels of IgG antibody. Case 4 is clearly a chronic case with high IgG in the absence of IgM antibody. Similarly cases 5 and 6 clearly conform with a diagnosis of acute brucellosis and cases 7 and 8 with chronic brucellosis.

It should be noted that all the first 8 cases have some positive serological finding using conventional tests. How do the latter correlate with the results of the radio-assay? Firstly, how does radio-assay of IgM antibody correlate with direct agglutination titres? Of the forty-six sera examined only nineteen showed significant titres (1 : 80 or above) in the direct agglutination test. Only one serum showed a

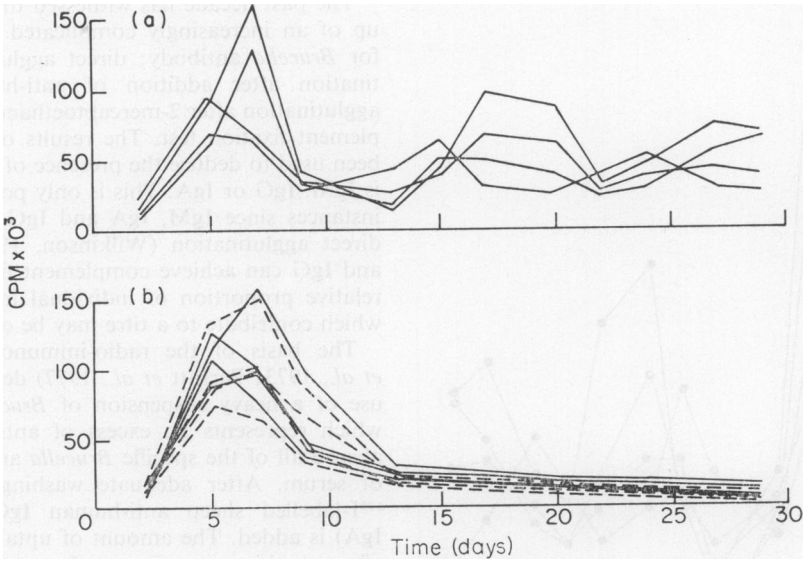

FIG. 6. (a) Antibody response to sheep red cells in individual fowls injected simultaneously with the two antigens 10^{10} sheep red cells and 10^{9} *Salmonella adelaide*. Response measured as 19S, IgM antibody by radioassay.

(b) Effect of an injection of 1 ml of a 7S antiserum at 9 days on the response to sheep red cells elicited as above. *Note* that passive 7S, IgG antibody to sheep red cells (dotted line) and passive 7S, IgG antibody to *S. adelaide* both secure feedback of the antibody response to sheep cells which is limited to a single peak. The repeated cycles of 19S antibody in the control birds (a) have been eliminated by passive treatment with 7S, IgG antibody of both specificities. — — — — Effect of passive 7S-anti-sheep red cells. ———— Effect of passive 7S-anti-*S. adelaide*. (Data from White and Nielsen, 1975.)

negative radio-assay and a direct agglutination titre of 1 : 80. In general, there was a very poor correlation between the radio-assay for IgM antibody and the direct agglutination. Seventeen sera with direct agglutination titres $< 1 : 80$ had raised concentrations of IgM antibody by radio-assay. Secondly, the indirect agglutination results correlated moderately well with IgG antibody concentrations determined by radio-assay. Similarly, radio-assay levels for IgG antibody correlated well with the titres obtained by complement fixation.

In Table 1, numbers 8–22 represent cases in which all conventional serological tests were negative. With cases 9, 12, 13, 16 and 20, the radio-assay results agree with conventional tests and a diagnosis of brucellosis can be excluded. In case 10, the radio-assay results indicate acute brucellosis as suggested by the increased IgM antibody level. The findings of case 14 indicate chronic brucellosis and those of case 15 indicate a low level of residual antibody, although it is atypical to find that this is IgM antibody. In cases 17, 18 and 19 the diagnosis of brucellosis is supported, and radio-assay reveals antibody undetected by the conventional tests. Case 21 (Payne, 1974) is very interesting in that repeated conventional tests in several different laboratories proved negative in spite of the isolation of *B. abortus* from the blood. The serum radio-assay indicated high concentrations of IgM and IgG antibody which confirm the relapse of a chronic brucellosis infection. Case 22 yielded radio-assay results (high IgA antibody) which were compatible with a diagnosis of chronic brucellosis.

It remains to be determined whether this quantitative and sensitive primary assay for *Brucella* antibody will prove useful for the diagnosis of a relapse during the course of chronic brucellosis. This could be signalled by a change from a low IgG antibody level to a rising peak of IgM with later IgG rise. The significance of the results might be greatly increased by the contemporaneous testing of sequential serum samples.

With conventional tests the diagnosis of brucellosis could never be excluded, even if the agglutination test, the mercaptoethanol test, the complement fixation test and the anti-human globulin findings were all negative (Payne, 1974). The use of a sensitive radio-assay which covers the primary reactivity of *Brucella* antibody of IgM, IgG and IgA classes

TABLE 1. The results obtained with this type of radio-immunoassay (RIA) in twenty-two patients suspected of brucellosis. The first eight cases have positive conventional tests indicating *Brucella* infection. Cases 9–22 have negative conventional tests for *Brucella* antibody

Case no.	Clinical data	RIA ct/min × 10^3 IgM	IgG	IgA	Direct agg. titre	Indirect agg. titre	CFT titre
1	Positive blood-culture of *B. abortus* 2 months previously	103	109	30	640	5120	256
2	Pyrexia 3 weeks	68	34	6	160	640	256
3	Vet pricked by inoculation needle	71	65	8	<20	2560	256
4	Farmer with night sweats and pyrexia. Son with brucellosis	4	140	10	5120	5120	256
5	Pyrexia with no obvious cause, of 2 weeks' duration	200	138	47	1280	1280	64
6	Dairy farmhand: pyrexia 2 weeks. Rash and joint pains	164	0	0	80	20	<4
7	Slaughterman with backache and febrile episodes	26	63	0	160	320	16
8	Malaise, giddiness, nausea, headache, and depression	27	63	0	1280	2560	256
9	Farmer: chronic ill health	4	13	0	<20	<20	<4
10	Farm dweller: intake of raw milk; painful hip	82	0	0	<20	<20	<4
11	Farmer's wife: pyrexia of uncertain cause: rigors and backache	53	0	14	<20	<20	<4
12	Veterinary staff: routine check	0	7	0	<20	40	4
13	Veterinary staff: routine check	0	29	0	20	40	8
14	Farmer with recurrent fever	24	15	58	<20	20	<4
15	Recovered from brucellosis	55	0	0	40	20	<4
16	Farmer: history of aches and pains	0	0	0	<20	<20	<4
17	Cowman: history of multiple complaints	25	75	27	<20	<20	4
18	Recurrent pyrexia	65	33	0	<20	<20	<4
19	Chronic brucellosis	113	28	0	80	40	<4
20	Farmer with 3rd episode of pyrexia of unknown origin	0	0	63	<20	<20	<4
21	*Brucella* sp. isolated from blood 1966. Negative serology. Several relapses. Possibly new relapse	84	70	0	<20	<20	<4
22	Herd manageress: recurrent fever	3	42	89	<20	40	8

should go a long way towards allowing the exclusion of a brucellosis diagnosis – always provided that the patient is not incapacitated by a severe immunological deficiency.

References

ANDERSON, R.K., JENNESS, R., BRUMFIELD, H.P. & GOUCH, P. (1964) Brucella-agglutinating antibodies: relation of mercaptoethanol stability to complement fixation. *Science*. New York, etc., **143**, 1334.

COGHLAN, J.D. & WEIR, D.M. (1967) Antibodies in human brucellosis. *British Medical Journal*, **2**, 269.

HENRY, C. & JERNE, N.K. (1967) The depressive effect of 7S antibody and the enhancing effect of 19S antibody in the regulation of the primary immune response. In: *Nobel Symposium No. 3: Gamma Globulins* (Ed. Killander, J.) p. 421. Almqvist & Wiksell, Stockholm.

ISHIZAKA, K. & OKUDAIRA, H. (1972) Reaginic antibody formation in the mouse. I. Antibody-mediated suppression of reaginic antibody formation. *Journal of Immunology*, **109**, 84.

KERR, W.R., COGHLAN, J.D., PAYNE, D.J.H. & ROBERTSON, L. (1966a) Chronic brucellosis in the practising veterinary surgeon. *Veterinary Record*, **79**, 602.

KERR, W.R., COGHLAN, J.D., PAYNE, D.J.H. & ROBERTSON, L. (1966b) The laboratory diagnosis of chronic brucellosis. *Lancet*, **ii**, 1181.

KERR, W.R., MCCAUGHEY, W.J., COGHLAN, J.D., PAYNE, D.J.H., QUAIFE, R.A., ROBERTSON, L. & FARRELL, I.D. (1968) Techniques and interpretations in the serological diagnosis of brucellosis in man. *Journal of Medical Microbiology*, **1**, 181.

KINCADE, P.W., & COOPER, M.D. (1971) Development and distribution of immunoglobulin-containing cells in the chicken. *Journal of Immunology*, **106**, 371.

KINCADE, P.W., LAWTON, A.R., BOCKMAN, D.E. & COOPER, M.D. (1970) Suppression of immunoglobulin G synthesis as the result of antibody-mediated suppression of immunoglobulin M synthesis in chickens. *Proceedings of the National Academy of Sciences of the United States of America*, **67**, 1918.

LAWTON, A.R., ASOFSKY, R., HYLTON, M.B. & COOPER, M.D. (1972) Suppression of immunoglobulin class synthesis in mice. I. Effects of treatment with antibody to μ-chain. *Journal of Experimental Medicine*, **135**, 277.

MANNING, D.D. & JUTILA, J.W. (1972a) Immunosuppression in mice injected with heterologous anti-immunoglobulin antisera. *Journal of Immunology*, **108**, 282.

MANNING, D.D. & JUTILA, J.W. (1972b) Immunosuppression of mice injected with heterologous anti-immunoglobulin heavy chain antisera. *Journal of Experimental Medicine*, **135**, 1316.

MARTIN, L.N. & LESLIE, G.A. (1974) IgM-forming cells as the immediate precursor of IgA-producing cells during ontogeny of the immunoglobulin-producing system of the chicken. *Journal of Immunology*, **113**, 120.

MÖLLER, G. & WIGZELL, H. (1965) Antibody synthesis at the cellular level. Antibody-induced suppression of 19S and 7S antibody response. *Journal of Experimental Medicine*, **121**, 969.

MURGITA, R.A., MATTIOLI, C.A. & TOMASI, T.B. (1973) Production of a runting syndrome and selective γA deficiency in mice by the administration of anti-heavy-chain antisera. *Journal of Experimental Medicine*, **138**, 209.

NIELSEN, K.H., PARRATT, D. & WHITE, R.G. (1973) Quantitation of antibody to particulate antigens using a radiolabelled anti-immunoglobulin reagent: application to estimation of antibody in farmer's lung syndrome. *Journal of Immunological Methods*, **3**, 301.

NIELSEN, K.H. & WHITE, R.G. (1974) Effect of host decomplementation on homeostasis of antibody production in fowl. *Nature*. London, **250**, 234.

PARRATT, D., NIELSEN, K.H., WHITE, R.G. & PAYNE, D.J.H. (1977) Radio-immunoassay of IgM, IgG and IgA *Brucella* antibodies. *Lancet*, **i**, 1075.

PAYNE, D.J.H. (1974) Chronic brucellosis. *British Medical Journal*, **2**, 221.

PIERCE, C.W., SOLLIDAY, S.M. & ASOFSKY, R. (1972) Immune response *in vitro*. IV. Suppression of primary γM, γG and γA plaque-forming cell responses in mouse spleen cell cultures by class-specific antibody to mouse immunoglobulins. *Journal of Experimental Medicine*, **135**, 675.

REDDIN, J.L., ANDERSON, R.K., JENNESS, R. & SPINK, W.W. (1965) The significance of 7S and macroglobulin *Brucella* agglutinins in human brucellosis. *New England Journal of Medicine*, **272**, 1263.

STRANNEGARD, Ö. & BERLIN, L. (1970) Suppression of reagin synthesis in rabbits by passively administered antibody. *Immunology*, **18**, 773.

UHR, J.W. & MÖLLER, G. (1968) Regulatory effect of antibody on the immune response. *Advances in Immunology*, **8**, 81.

WALKER, J.G. & SISKIND, G.W. (1968) Effect of antibody affinity upon its ability to suppress antibody formation. *Immunology*, **14**, 21.

WHITE, R.G. & NIELSEN, K.H. (1975) Interactions between the immunological responses of a thymus-independent antigen (*Salmonella adelaide* O antigen) with a thymus-dependent antigen (sheep erythrocytes) in the adult bird. *Immunology*, **28**, 959.

WILKINSON, P.C. (1966) Immunoglobulin patterns of antibodies against *Brucella* in man and animals. *Journal of Immunology*, **96**, 457.